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Intestinal lactobacilli and the *DC-SIGN* gene for their recognition by dendritic cells play a role in the aetiology of allergic manifestations

John Penders,[1,2] Carel Thijs,[2] Monique Mommers,[2] Ellen E. Stobberingh,[1] Edward Dompeling,[3] Naomi E. Reijmerink,[4,5] Piet A. van den Brandt,[6] Marjan Kerkhof,[7] Gerard H. Koppelman[8] and Dirkje S. Postma[4]

Correspondence
John Penders
j.penders@mumc.nl

[1]Department of Medical Microbiology, Care and Public Health Research Institute (Caphri) Maastricht University Medical Centre, 6202 AZ Maastricht, The Netherlands

[2]Department of Epidemiology, Care and Public Health Research Institute (Caphri), Maastricht University, Maastricht, The Netherlands

[3]Department of Pediatric Pulmonology, Maastricht University Medical Centre, Maastricht, The Netherlands

[4]Department of Pulmonology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[5]Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[6]Department of Epidemiology, Research Institute Growth and Development (GROW), Maastricht University, Maastricht, The Netherlands

[7]Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, Groningen, The Netherlands

[8]Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Diminished exposure to harmless micro-organisms, such as lactobacilli, has been suggested to play a role in the increased prevalence of allergic disorders in Westernized communities. The development of allergies depends on both environmental factors and genetic variations, including polymorphisms in genes encoding pattern recognition receptors. The present study examines the effects of both colonization with specific *Lactobacillus* species and genetic variations in DC-SIGN, a pattern recognition receptor on dendritic cells that recognizes lactobacilli, on the development of atopic dermatitis (AD) and sensitization in infancy. Within the KOALA Birth Cohort Study, faecal samples of 681 one-month-old infants were collected and quantitatively screened for five *Lactobacillus* species: *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. acidophilus* and *L. reuteri*. Eleven haplotype-tagging polymorphisms in the *DC-SIGN* gene were genotyped in these children. Allergic outcomes were a clinical diagnosis of AD and sensitization (specific IgE) at age 2 years. *L. rhamnosus* (31.5 %), *L. paracasei* (31.3 %) and *L. acidophilus* (14.4 %) were frequently detected in the faecal samples of one-month-old infants, whereas *L. casei* (2.5 %) and *L. reuteri* (<1 %) were rare. Colonization with *L. paracasei* decreased the risk of AD significantly (odds ratio 0.57, 95 % confidence interval 0.32–0.99), whereas effects of *L. acidophilus* were of borderline statistical significance (0.46, 0.20–1.04). Two *DC-SIGN* polymorphisms, rs11465413 and rs8112555, were statistically significantly associated with atopic sensitization. The present study supports the 'old friends' hypothesis suggesting that certain health-beneficial micro-organisms protect us from developing allergies and that these protective effects are species-dependent. Firm conclusions on the potential interaction between lactobacillus colonization and genetic variations in *DC-SIGN* in association with the development of allergic disorders cannot be drawn, given the limited power of our study. Therefore, incorporation of consecutive faecal sampling in newly started (birth) cohort studies would be a first requisite to further increase our understanding of host–microbial interactions in health and disease.

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Abbreviations: AD, atopic dermatitis; CI, confidence interval; DC, dendritic cell; DC-SIGN, DC-specific ICAM-3-grabbing non-integrin; LD, linkage disequilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism.

INTRODUCTION

The 'old friends' hypothesis is one of the most popular explanations for the increase in prevalence of asthma and allergies in the past decades. It implies that the increase in allergic (atopic) disorders in Westernized communities is (partly) explained by diminished exposure to harmless micro-organisms ('old friends') like lactobacilli (Rook *et al.*, 2004; Rook & Brunet, 2005). These micro-organisms are thought to prime immune regulation. Particularly, the modulation of antigen-presenting cell function by these micro-organisms and consequently the induction of regulatory T (T_{reg}) cells are considered important (Boirivant & Strober, 2007; Rook & Brunet, 2005). Dendritic cells (DCs) are antigen-presenting cells that play an essential role in mucosal tolerance. Depending on the microbial stimulus encountered, DCs secrete cytokines that dictate specific differentiation of unprimed, naïve $CD4^+$ T cells toward Th1, Th2, Th17 or T_{reg} cell responses (Geijtenbeek *et al.*, 2009; Mohamadzadeh *et al.*, 2005).

Epidemiological evidence for a protective effect of indigenous lactobacilli on allergic disorders is inconsistent. The first study comparing intestinal microbiota of 2-year-old children with and without atopic dermatitis (AD) showed a lower prevalence of lactobacilli in the faeces of children with the disease (Björkstén *et al.*, 1999). Perturbation in the gut microbiota of allergic children was confirmed in many subsequent studies, but none of these studies showed differences in the prevalence of intestinal lactobacilli (Penders *et al.*, 2007a). The quantification of lactobacilli within these studies was limited to the genus level. As different species of lactobacilli induce distinct and even opposing immune responses (Christensen *et al.*, 2002), the potential species-specific effects of lactobacilli in the aetiology of allergic disorders may thus have been overlooked. Indeed a recent study that differentiated between group I and II lactobacilli reported that children who developed allergy were significantly less colonized with group I lactobacilli (*L. rhamnosus, L. paracasei* and *L. casei*) during their first months of life (Sjögren *et al.*, 2009).

However, the development of allergic diseases depends not only on environmental factors (like microbial stimulation) but also on genetic factors. Genes involved in microbial recognition, binding and subsequent immune signalling may be important candidates in this respect. The C-type lectin receptor DC-specific ICAM-3-grabbing non-integrin (DC-SIGN) is of special interest regarding the recognition and binding of *Lactobacillus* species. Smits *et al.* (2005) showed that *L. reuteri* and *L. casei*, but not *L. plantarum*, bind to DC-SIGN and prime monocyte-derived DCs to drive the development of IL-10-producing T_{reg} cells. Recently, it was demonstrated that *L. acidophilus* NCFM (North Carolina Food Microbiology) attaches to DCs and induces a concentration-dependent production of IL-10 and low IL12p70. It was further shown that the cellular contacts of DCs and *L. acidophilus* involve a specific interaction between DC-SIGN and surface layer protein A of this species (Konstantinov *et al.*, 2008).

Genetic variations in the DC-SIGN receptor have been shown to influence the susceptibility to several infectious agents such as *Mycobacterium tuberculosis* (Selvaraj *et al.*, 2009; Vannberg *et al.*, 2008), human immunodeficiency virus-1 (Liu *et al.*, 2004; Martin *et al.*, 2004; Selvaraj *et al.*, 2009) and dengue virus (Sakuntabhai *et al.*, 2005). To our knowledge, no studies have so far examined variations in *DC-SIGN* in association with allergic diseases.

To gain more insight into the species-specific effects of indigenous lactobacilli on the aetiology of allergic diseases, we studied the association between colonization with specific *Lactobacillus* species in early infancy and the development of AD and allergic sensitization in the first 2 years of life. Furthermore, we assessed effects of genetic variations in *DC-SIGN* on the development of these atopic outcomes.

METHODS

Design. This study was conducted within the KOALA Birth Cohort Study, a prospective cohort in the Netherlands, described in detail elsewhere (Kummeling *et al.*, 2005). Briefly, 2834 pregnant women were recruited at 34 weeks of gestation. During pregnancy and early childhood, data on perinatal determinants of the child's health as well as on hygiene, infections, nutrition, child rearing and other lifestyle characteristics were collected for all members of the cohort by repeated questionnaires at 34 weeks of gestation and 3, 7, 12 and 24 months post-partum. The study was approved by the medical ethics committee of Maastricht University. Written informed parental consent was obtained for all participants.

Recruitment of the subcohort. This study investigated all children for whom both a faecal sample, at age 1 month, and a buccal swab sample were available.

All participating parents were asked to provide a buccal swab sample of their child for genotyping, whereas only those participants recruited from January 2002 onwards were asked to consent to sample their child's faeces. After exclusion of premature infants, infants receiving antimicrobial agents during their first month of life, or those having insufficient or no faeces (<1 g) collections between the ages of 3 and 6 weeks post-partum, and infants without faeces questionnaires, 681 children were eligible for analyses.

When the children reached the age of 2 years, the parents of 506 of the 681 children consented to a home visit for a physical examination of the child for AD and the collection of venous blood samples to determine specific and total IgE levels.

Faecal microbiota composition. The collection and processing of faecal samples has been described in detail elsewhere (Penders *et al.*, 2007b). Briefly, parents collected a faecal sample by placing a sanitary napkin in the diaper (to prevent absorption of the faeces by the diaper), collected the faeces out of the napkin into the collection tube and sent it immediately to our laboratory by post. At the laboratory faecal samples were diluted tenfold in peptone-water (Oxoid CM0009) containing 20 % (v/v) glycerol (Merck) and stored at –20 °C until analysis. DNA was extracted by a combination of bead-beating and the QIAamp DNA Stool Mini kit. For the present study, faecal samples were subjected to real-time PCRs for the enumeration of total lactobacilli, *L. casei*, *L. paracasei*, *L. reuteri*, *L. rhamnosus* and *L. acidophilus*.

L. paracasei, *L. acidophilus* and *L. rhamnosus* were selected because of their relatively high prevalence in this age group compared to other *Lactobacillus* species (Ahrné *et al.*, 2005; Haarman & Knol, 2006; Mitsou *et al.*, 2008; Savino *et al.*, 2005). *L. casei* and *L. reuteri* were less prevalent in these studies, but were additionally selected because of their proven interaction with DC-SIGN (Smits *et al.*, 2005).

For the quantitative detection of *L. reuteri*, *L. casei* and *L. paracasei*, 5′-nuclease assays were used as described by Haarman & Knol (2006) with slight modifications in primer and probe concentrations. Briefly, amplifications were conducted in a total volume of 25 µl, containing 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 300 nM of both primers, 100 nM TaqMan MGB-probe, and 2 µl purified target DNA. The amplification (2 min at 50 °C, 10 min at 95 °C, followed by 42 cycles of 15 s at 95 °C and 1 min at 60 °C) and detection were conducted with the ABI Prism 7000 sequence detection system.

For the quantification of *L. acidophilus, L. rhamnosus* and total lactobacilli, real-time detection of PCR products was conducted with SYBR Green I (primers listed in Table 1). For *L. acidophilus* and *L. rhamnosus*, amplifications were conducted in a total volume of 25 µl, containing 1× iQ SYBR Green Supermix (Bio-Rad), 2 µl purified target DNA and 100 nM of both primers for *L. rhamnosus* or 300 nM of both primers for *L. acidophilus*. The amplification (5 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 15 s, binding and extension for 1 min at 62 °C for *L. rhamnosus* or at 60 °C for *L. acidophilus*) and detection were conducted with the MyiQ Single-colour real-time PCR Detection System (Bio-Rad). For total lactobacilli, amplifications were conducted in a total volume of 25 µl, containing 1× iQ SYBR Green Supermix (Bio-Rad), 5 µl purified target DNA and 500 nM of both primers. The amplification was conducted as follows: 5 min at 95 °C, followed by 35 cycles consisting of 15 s at 95 °C, 20 s at 58 °C, 45 s at 72 °C, with a final extension step at 72 °C for 5 min. Following amplification, melt curve analysis was performed from 60 to 95 °C using increments of 0.5 °C per 10 s.

Samples were considered to be positive when threshold cycle (C_t) values were below 35 for total lactobacilli and below 42 for the species-specific PCRs .

Measurement of infants' specific IgE. Venous blood samples were collected from the infants during a home visit at 2 years post-partum. They were analysed for specific IgE against hen's eggs, cow's milk, peanuts, birch, grass pollen, cat, dog and house dust mite using a radioallergosorbent test (RAST) as described earlier (Aalberse *et al.*, 1981). The detection limit for specific IgE was 0.10 IU ml^{-1}.

Definition of atopic dermatitis (AD) and sensitization. Children were defined as having AD according to the UK Working Party (UK-WP) criteria (Williams *et al.*, 1994) as determined by a trained research nurse during the home visit at the age of 2 years. The presence of AD was diagnosed from the presence of four clinical symptoms: 1, presence of itchy rash; 2, history of flexural dermatitis; 3, visible flexural dermatitis; and 4, onset before the age of 2 years. As described previously (Penders *et al.*, 2007b), infants with a UK-WP probability score of AD >90 % were regarded as having AD.

Infants were classified as having allergic sensitization when serum IgE levels were >0.3 IU ml^{-1} for one or more of the tested food or inhalant allergens.

DNA collection and processing, SNP selection and genotyping. Parents were asked to collect buccal swabs, as source of DNA, from their children. Genomic DNA was extracted from these swabs by standard methods (Sambrook & Russell, 2001). DNA was amplified by using REPLI-g UltraFast technology (Qiagen).

Haplotype-tagging SNPs were selected from the publicly available database on the Innate Immunity website (Lazarus *et al.*, 2002). Genotyping was performed by competitive allele-specific PCR using KASPar genotyping chemistry, performed under contract by K-Biosciences with extensive quality control as described previously (Bottema *et al.*, 2008).

Statistical analysis. The prevalence of AD and allergic sensitization was calculated according to colonization with the specific *Lactobacillus* species. χ^2 tests were used to test for differences in the prevalence of the atopic outcomes between colonized and uncolonized subjects.

Table 1. Primers and probes used in this study

Target organism (amplicon size)	Primer/probe	Sequence (5′–3′)	T_m (°C)	Reference
Lactobacillus reuteri (93 bp)	Forward primer	ACCGAGAACACCGCGTTATTT	59	Haarman & Knol (2006)
	Reverse primer	CATAACTTAACCTAAACAATCAAAGATTGTCT	59	
	Probe	6-FAM-ATCGCTAACTCAATTAAT-MGBNFQ	69	
Lactobacillus acidophilus (124 bp)	Forward primer	GATCGCATGATCAGCTTATA	52	Furet *et al.* (2004)
	Reverse primer	AGTCTCTCAACTCGGCTATG	53	
Lactobacillus rhamnosus (116 bp)	Forward primer	TGCTTGCATCTTGATTTAATTTTG	52	Byun *et al.* (2004)
	Reverse primer	GGTTCTTGGATYTATGCGGTATTAG	54	
Lactobacillus paracasei (80 bp)	Forward primer	ACATCAGTGTATTGCTTGTCAGTGAATAC	60	Haarman & Knol (2006)
	Reverse primer	CCTGCGGGTACTGAGATGTTTC	60	
	Probe	6-FAM- TGCCGCCGGCCAG-MGBNFQ	70	
Lactobacillus casei (132 bp)	Forward primer	CTATAAGTAAGCTTTGATCCGGAGATTT	59	Haarman & Knol (2006)
	Reverse primer	CTTCCTGCGGGTACTGAGATGT	59	
	Probe	6-FAM-ACAAGCTATGAATTCACTTGC-MGBNFQ	70	
Lactobacillus spp. (341 bp)	Forward primer	AGCAGTAGGGAATCTTCCA	59	Rinttilä *et al.* (2004)
	Reverse primer	CACCGCTACACATGGAG	59	

Linkage disequilibrium (LD) was calculated using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview), by determining D' and r^2 values. Genotype distribution was tested for deviations ($P<0.05$) from Hardy–Weinberg equilibrium using χ^2 analyses.

We used χ^2 tests to analyse whether *DC-SIGN* SNPs were associated with AD and allergic sensitization by using a co-dominant model.

Unadjusted and adjusted odds ratios (ORs) and 95 % confidence intervals for the association between *Lactobacillus* species colonization and atopic outcomes according to the different genotypes were calculated by means of logistic regression. The adjusted regression models included the following potential confounders: 1, parental atopic history, defined as self-reported doctor's diagnosed eczema, hay fever, asthma, pet and/or house dust mite allergy (both parents non-atopic; at least one parent atopic); 2, siblings atopic history, defined as parentally reported doctor's diagnosed food allergy, eczema, hay fever, asthma, pet and/or house dust mite allergy (no siblings; $\geqslant$1 siblings, all none-atopic; or $\geqslant$1 siblings, at least one atopic); 3, age at collection of faecal sample (age in days); 4, infant's sex (boy; girl); 5, pet exposure at home (no pet exposure; only cat exposure; only dog exposure; only other furry pet exposure; combination of cat, dog and/or other furry pet exposure); 6, recruitment group (alternative; conventional) (Kummeling *et al.*, 2005).

To test whether the effect of *Lactobacillus* species was modified by host genotype, an interaction term of genotype and *Lactobacillus* species colonization was included in the logistic regression models and compared to the models without the interaction terms using the likelihood ratio test. All analyses were performed using SPSS 15.0 statistical software and results were considered statistically significant at $P<0.05$. To control for multiple testing, we calculated the false discovery rate (FDR) for the tests for interaction (based on 33 tests for interaction per outcome) according to Benjamini & Hochberg (1995). The *P*-values were tested for significance at a FDR (*q*-value) of 0.05.

RESULTS

Intestinal colonization by lactobacilli and allergic manifestations

The prevalence of the *Lactobacillus* species in the current study population was as follows: *L. rhamnosus* 31.5 %, *L. paracasei* 31.3 %, *L. acidophilus* 14.4 %, *L. casei* 2.5 % and *L. reuteri* <1 %. Because of the low prevalence of *L. casei* and *L. reuteri* in our population, these bacteria were not taken into account for further analysis.

Information on AD was available for all 506 children that were visited at home, whereas information on atopic sensitization was available for 492 children.

The risk of AD at age 2 years was significantly lower in children who were colonized with *L. paracasei* [odds ratio (OR) 0.57, 95 % confidence interval (CI) 0.32–0.99, $P=0.04$] compared with those not colonized. For *L. acidophilus* a borderline statistically significant decreased risk of AD was found (OR 0.46, 95 % CI 0.20–1.04) ($P=0.06$). Neither of these species, nor *L. rhamnosus*, were however associated with the risk of atopic sensitization (Table 2).

DC-SIGN gene variants

Eleven *DC-SIGN* SNPs were genotyped (Table 3), all of which were in Hardy–Weinberg equilibrium. Strong LD, with D' approaching the value of 1 (Fig. 1), was observed for rs7252229, rs2287886, rs4804803, rs735239, rs735240, rs11465360, rs7359874 and rs4804804.

Table 4 presents the association between these SNPs and the atopic outcomes under study. None of the SNPs were associated with AD. Two SNPs, rs11465413 and rs8112555, were statistically significantly associated with atopic sensitization. Of the children who were heterozygous for rs11465413, 17.9 % were sensitized at the age of 2 years as compared to 30.0 % of the homozygotes for the major allele ($P=0.04$). Heterozygotes for rs8112555 were also at decreased risk of developing atopic sensitization as

Table 2. Prevalence of allergic sensitization and AD according to *Lactobacillus* species colonization

Lactobacillus	Present?	Sensitization % (cases/total)	AD* % (cases/total)
L. paracasei	No	28.4 (95/334)	18.1 (62/343)
	Yes	28.0 (44/157)	11.1 (18/162)
		$P=0.92$	**$P=0.04$**
L. rhamnosus	No	27.3 (92/337)	15.3 (53/347)
	Yes	30.1 (46/153)	17.2 (27/157)
		$P=0.53$	$P=0.58$
L. acidophilus	No	28.0 (116/415)	17.1 (73/426)
	Yes	31.2 (24/77)	8.8 (7/80)
		$P=0.57$	$P=0.06$
Lactobacillus spp.	No	28.0 (94/336)	15.7 (54/345)
	Yes	29.5 (46/156)	16.1 (26/161)
		$P=0.73$	$P=0.89$

*AD according to UK Working Party criteria.

Table 3. Selected *DC-SIGN* SNPs and minor allele frequencies in the study population

DC-SIGN SNP	Alleles*	Minor allele frequency	*P*-value† for deviation from Hardy–Weinberg equilibrium
rs11465360	C : A	0.05	0.39
rs11465413	T : A	0.09	0.42
rs11465421	A : C	0.47	0.35
rs2287886	G : A	0.36	0.51
rs4804803	A : G	0.19	0.57
rs4804804	G : A	0.36	0.41
rs7252229	G : C	0.13	0.96
rs735239	A : G	0.34	0.86
rs735240	G : A	0.44	0.86
rs7359874	A : T	0.49	0.45
rs8112555	G : A	0.14	0.84

*Minor alleles first.
†As determined by χ^2 test.

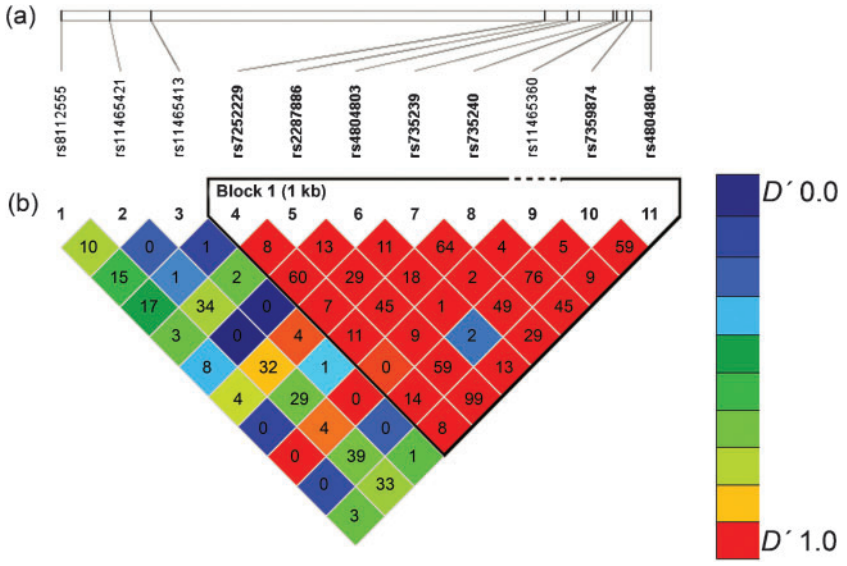

Fig. 1. Position and linkage disequilibrium of the SNPs within *DC-SIGN*. (a) *DC-SIGN* is located on chromosome 19 (19p13). Eleven haplotype tagging *DC-SIGN* SNPs were selected. All SNPs are in Hardy–Weinberg (HW) equilibrium ($P>0.05$). (b) Pairwise linkage disequilibrium plot according to Haploview 4.1 for DC-SIGN SNPs. D' is represented by colour, changing from red ($D'=1$) to dark blue ($D'=0$). D' is 1 when no recombination has occurred between two SNPs. The measure of r^2 is represented by a number ranging from 0 to 100. When the minor alleles at two SNP positions are always present on the same haplotype, $r^2=100$; when the minor alleles are always on separate haplotypes, $r^2=0$.

compared to children homozygotic for the major alleles of this SNP ($P=0.03$).

Gene–environment interaction

To examine whether the associations between colonization with the *Lactobacillus* species and atopic outcomes were modified by genetic variations in *DC-SIGN*, we examined these associations stratified for *DC-SIGN* genotype, and tested for statistical interaction (test for differences of odds ratios between strata of *DC-SIGN* genotypes). The only statistically significant interaction (P for interaction=0.03) was found between *L. rhamnosus* colonization and rs11465360. *L. rhamnosus* colonization was associated with an increased risk of AD in homozygotes for the major allele (CC) (OR 1.63, 0.93–2.84) and with a decreased risk in heterozygotes (CA) (OR 0.17, 0.02–1.35); however, this interaction did not withstand correction for multiple testing.

DISCUSSION

We examined the effect of common intestinal *Lactobacillus* species in early infancy and genetic variations in *DC-SIGN* in association with development of AD and allergic sensitization in early childhood within the KOALA birth cohort.

Colonization by *L. acidophilus* was associated with a statistically significant decreased risk of AD, whereas effects of *L. paracasei* were of borderline significance. These findings are in agreement with the 'old friends' hypothesis suggesting that certain health-beneficial micro-organisms may protect us from developing allergies and that these effects are species-dependent.

However, we did not find any effect of *L. acidophilus* and *L. paracasei* colonization on atopic sensitization.

No previous observational studies have examined the association of specific *Lactobacillus* species, but the effects of *L. paracasei* and *L. acidophilus* supplementation on the primary prevention of (atopic) dermatitis have been studied in randomized controlled trials. In agreement with our study, the trial by West *et al.* (2009) demonstrated that *L. paracasei* supplementation in early infancy reduced the incidence of eczema but did not influence atopic sensitization. Indeed, the role of allergic sensitization in childhood AD remains far from clear. It is neither a prerequisite nor a uniform cause of the disease (Williams & Flohr, 2006).

In contrast to the above results, however, *L. acidophilus* (LAVRI-A1) supplementation in high-risk newborns failed to reduce the incidence of eczema as compared to placebo (Taylor *et al.*, 2007).

As a pattern recognition receptor, DC-SIGN interacts with a plethora of, mainly pathogenic, micro-organisms other than lactobacilli (Geijtenbeek *et al.*, 2000). DC-SIGN is organized into three domains: an N-terminal cytoplasmic region, a neck region containing eight repeats of a 23 aa sequence, and a C-terminal C-type lectin domain (Soilleux *et al.*, 2000). The *CD209* gene is located on chromosome 19p13.2-3 and is highly polymorphic.

In the present study, two *DC-SIGN* SNPs (rs11465413 and rs8112555) were associated with atopic sensitization. Homozygotes for the major allele of these SNPs had a significantly higher risk of sensitization than heterozygous children. Because of the low minor allele frequency of both SNPs, the risk of sensitization in children homozygous for the minor allele could not be examined.

Since this is the first study in which the association between *DC-SIGN* gene variants and atopic disorders has been studied, it would be worthwhile to examine whether these associations could be confirmed in another population. To

Table 4. Prevalence of allergic sensitization and AD at age 2 years according to *DC-SIGN* genotype

***DC-SIGN* genotype**		**Allergic sensitization % (cases/total)**	**AD % (cases/total)**
rs11465360	CC	27.9 (120/430)	15.9 (70/441)
	CA	29.3 (12/41)	20.9 (9/43)
	AA*	50.0 (1/2)	0 (0/2)
		P=0.85	*P*=0.39
rs11465413	TT	30.0 (124/413)	14.9 (63/423)
	TA	17.9 (12/67)	19.7 (14/71)
	AA	* (1/3)	* (1/3)
		***P*=0.04**	*P*=0.30
rs11465421	AA	29.7 (44/148)	15.2 (23/151)
	CA	27.7 (62/224)	14.6 (34/233)
	CC	28.3 (30/106)	18.5 (20/108)
		P=0.91	*P*=0.64
rs2287886	GG	27.1 (54/199)	15.8 (32/203)
(CD209 139 C/T)	GA	29.0 (63/217)	16.5 (37/224)
	AA	29.7 (19/64)	21.1 (8/66)
		P=0.88	*P*=0.69
rs4804803	AA	27.7 (88/319)	14.9 (49/328)
(CD209 336 C/T)	GA	29.6 (42/142)	15.1 (22/146)
	GG	31.8 (7/22)	31.8 (7/22)
		P=0.86	*P*=0.11
rs4804804	GG	27.6 (56/203)	15.5 (32/207)
(CD209 1466 C/T)	GA	28.2 (61/216)	17.0 (38/224)
	AA	29.2 (19/65)	11.9 (8/67)
		P=0.97	*P*=0.61
rs7252229	GG	28.9 (105/363)	14.2 (53/373)
(CD209 216 C/G)	CG	23.4 (25/107)	18.9 (21/111)
	CC	* (5/9)	* (5/9)
		P=0.26	*P*=0.23
rs735239	AA	29.5 (62/210)	17.5 (38/217)
(*CD209 871 C/T*)	GA	27.6 (56/203)	13.9 (29/208)
	GG	27.0 (17/63)	14.1(9/64)
		P=0.88	*P*=0.56
rs735240	GG	30.7 (46/150)	17.5 (27/154)
(CD209 939 C/T)	GA	27.0 (61/226)	13.7 (32/234)
	AA	28.4 (27/95)	15.5 (15/97)
		P=0.74	*P*=0.59
rs7359874	TT	31.9 (38/119)	17.9 (22/123)
(CD209 1180 A/T)	TA	25.6 (58/227)	14.9 (35/235)
	AA	30.2 (39/129)	13.0 (17/131)
		P=0.40	*P*=0.55
rs8112555	GG	30.5 (110/361)	15.9 (59/371)
(CD209 7882 C/T)	GA	19.6 (22/112)	13.8 (16/116)
	AA	* (5/8)	* (3/8)
		***P*=0.03**	*P*=0.58

*Minor allele homozygotes excluded from χ^2 tests analyses because of limited number of subjects.

our knowledge, studies in which our findings could be replicated are currently lacking. It would therefore be of high value if newly conducted birth cohort studies on allergic, but also other, diseases were to incorporate consecutive faecal sampling throughout the first year of life. As well as replication of our findings, this could significantly enhance the possibilities and power to study host–microbial interaction in health and disease by pooling data from such studies. In the current molecular era, incorporation of faecal sampling within such new observational studies can be achieved relatively easily.

Within the present study no statistically significant gene-by-environment interactions were found after adjusting for

multiple testing. This may partly be due to the relatively low number of children with both DNA and faecal samples available, even though this is one of the largest, if not the largest, cohort on gut microbial ecology. Furthermore, the low minor allele frequency of this particular SNP also reduced the power to detect an interaction that could withstand correction for multiple testing. Whether the effects of *Lactobacillus* species on the development of atopic diseases are modified by genetic variations in *DC-SIGN* can therefore not be answered until sample sizes can be increased significantly by pooling data from new studies.

The present study was limited in the number of *Lactobacillus* species that were targeted: we only examined five of the 17 *Lactobacillus* species that can be present in the human gastrointestinal tract (Walter, 2008). However, we do not expect to have missed any species that are commonly found in early infancy. Our results on the *Lactobacillus* species composition are in agreement with several previous studies which consistently showed that around the age of 1 month *L. paracasei*, *L. acidophilus* and *L. rhamnosus* (Ahrné *et al.*, 2005; Haarman & Knol, 2006; Mitsou *et al.*, 2008; Savino *et al.*, 2005) are among the most prevalent species. In the same studies *L. reuteri* and *L. casei* and several other species were absent or contributed only a tiny amount to the *Lactobacillus* microbiota of infants (Ahrné *et al.*, 2005; Haarman & Knol, 2006; Mitsou *et al.*, 2008; Savino *et al.*, 2005).

Another drawback of our study is the availability of only one faecal sample per subject. Caution is therefore due when particular *Lactobacillus* species are considered as lasting (autochthonous) inhabitants. It has so far not been confirmed that the *Lactobacillus* species examined in the present study actually form stable populations. Therefore, the presence of these species may only indicate the passage of allochthonous strains. As a consequence, our results may not be directly comparable with results of clinical trials in which continuous exposure to the probiotic micro-organism(s) under study during the intervention period is assumed to take place at relevant immune surfaces in the gastrointestinal tract. It is therefore plausible that similar interactions between the host's genotype and probiotics exist and that this could at least partly explain differences in the results of probiotic trials. It would therefore be of interest to study the effects of probiotic trials within specific genetic subgroups. This may also partly resolve inconsistencies between trials in populations with different distributions of genetic variants.

In conclusion, the current study shows a decreased risk of AD in children colonized by *L. acidophilus* and *L. paracasei* and provides evidence that there is an association between two *DC-SIGN* SNPs and allergic sensitization.

To further increase our understanding of host–microbial interactions in health and disease, the incorporation of consecutive faecal sampling in newly developed (birth) cohort studies would be a first requisite.

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